

Infection of American Bullfrog Tadpoles, *Lithobates catesbeianus* (Shaw), by Anchor Worm, *Lernaea cyprinacea* L., in Streams of Northeastern Ohio

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Abstract: In some years, the copepod parasite *Lernaea cyprinacea* L. (Anchor Worm) parasitizes fishes in the Grand River, Conneaut Creek, and the Ashtabula River of northeastern Ohio. Here, I report on the occurrence and prevalence of this parasite on *Lithobates catesbeianus* (Shaw) (American Bullfrog) tadpoles at sites in reaches of these streams.

Keywords: *Lernaea cyprinacea*, Anchor Worm, *Lithobates catesbeianus*, American Bullfrog, amphibian parasite

Introduction

The copepod *Lernaea cyprinacea* L. (Anchor Worm) is primarily an opportunistic ectoparasite of freshwater fishes (Demaree 1967, Eisen 1977, Enders and Rifenburgh 1928, Wilson 1914). It also infects amphibians, primarily tadpoles (Baldauf 1961; Hoffman 1976; Kupferberg et al. 2009; Ming 2001; Tidd 1934, 1962), caudates including *Dicamptodon ensatus* (Eschscholtz) (Kupferberg et al. 2009) and the axolotl *Amybystoma mexicanum* (Shaw and Nodder) (Carnevia and Speranza 2003), and several orders of insects (McAllister et al. 2011). *Lernaea cyprinacea* is a Eurasian species that has become widespread globally through the introduction of fishes, often via the aquarium trade (Tidd 1934) or fish culture (Enders and Rifenburgh 1928, Hoffman 1976). The infection is usually first recognized through observation of barbel-like filaments extending from various locations on the body of the host. In fish, the copepods typically anchor on or at a fin base (Whitaker and Schlueter 1975, Wilson 1914; pers. obs.). In amphibians, *L. cyprinacea* may embed posterior to the papillae of the mouth (Baldauf 1961); in or near the groove of the body and tail juncture, especially near the vent and the origin of the hind legs (Alcalde and Batistoni 2005, Kupferberg et al. 2009, Tidd and Shields 1963); or near the spiracle (Bird 1968; Shields and Tidd 1968.).

The life cycle of *L. cyprinacea* includes three naupliar free-swimming larval stages (two nauplii and a metanauplii), followed by five parasitic copepodid stages. Larval entry into the tadpole host is through the spiracle (Bird 1968, Shariff and Somerville 1986); the parasitic larvae attach inside the mouth or to gill filaments within the branchial chamber (Shields and Tidd 1963) or to the integument (Tidd 1970). Sexual differentiation and copulation occur during the fifth copepodid stage. Following copulation, the males die within 24 h, whereas the female moves to the exterior surface integument, attaches, and feeds on the mucosa of the host (Shariff and Somerville 1986, Shields and Tidd 1963, Tidd 1970). She then penetrates the integument and undergoes metamorphosis, during which the body segments elongate and the cephalothorax transforms and develops four horns, two of which are branched (Enders and Rifenburgh 1928). The horns embed and anchor the female in the integument, in muscle, and often in organs within the coelom (Tidd and Shields 1963). Two egg sacs develop on the distal end of the elongated parasite; they may each contain 50–250 eggs (Shields and Goode 1978, Streckler and Yanong 2017) and are replaced multiple times (Fig. 1). The entire life cycle requires only 18–25 days at water temperatures of 25–30 °C and is temperature dependent (Grabda 1963, Streckler and Yanong 2017). Development is slowed in all stages at lower temperatures (<20 °C; Shields and Tidd 1968) and the overwintering population consists of transformed females (Nakai and Kokai 1931, Shields and Tidd 1968). Infections of native species *Lithobates catesbeianus* (American Bullfrog) and *Lithobates clamitans* (Latreille) (Green Frog) tadpoles by *L. cyprinacea* in streams of central Ohio were first described by Tidd (1962, 1970) and Tidd and Shields (1963). Fishes infected with *L. cyprinacea* were netted at numerous sites over the years in northeastern Ohio stream drainage systems; consequently, the copepod is widely distributed in these streams and is environmentally available to infect amphibians. Here, based upon long-term observations, I report on infections of amphibian tadpoles by *L. cyprinacea* in reaches of three streams in northeastern Ohio.



Figure 1. Adult female *Lernaea cyprinacea*, illustrating the anterior anchor (left) and two posterior egg sacs (right).

Methods

Field observations of *L. catesbeianus* tadpoles were made in the Grand River, Ashtabula River, and Conneaut Creek of Lake and Ashtabula counties of northeast Ohio while conducting vertebrate and crayfish surveys from 1993–2017 (Fig. 2). During an amphibian population study in the Grand River in 1993, numerous second-year *L. catesbeianus* tadpoles were netted, examined, and found to be infected with mature female *L. cyprinacea*. Thereafter, netted tadpoles in reaches of all northeast Ohio streams were examined for lernaeids from July through early September. Sampling effort to net and examine tadpoles was not evenly distributed between sites. Site 1 in both the Grand and Ashtabula rivers were sites visited repeatedly during population studies of *Necturus*. Other sites were sampled once annually on various dates or irregularly over the years, the presence of *L. catesbeianus* and *Lernaea* having been detected during earlier fish surveys. Beginning in 1999, the number of *L. cyprinacea* and their attachment positions were recorded. Parasite prevalence (number of infected tadpoles/total number of tadpoles inspected) was calculated for sites in years when tadpoles were encountered. Morphological abnormalities of host tadpoles were noted in the field, and eight parasitized tadpoles or metamorphosing individuals with morphological abnormalities were transported to the lab and reared to determine whether metamorphosis would proceed normally. Tadpoles were reared in dechlorinated tap water in a 20-gallon (85 L) aquarium at room temperature ($21^{\circ}\text{C} \pm 2^{\circ}\text{C}$) under 8 h of fluorescent lighting supplemented with plant grow lights; they were fed tetra fish food and algae pellets twice weekly until metamorphosis was complete.

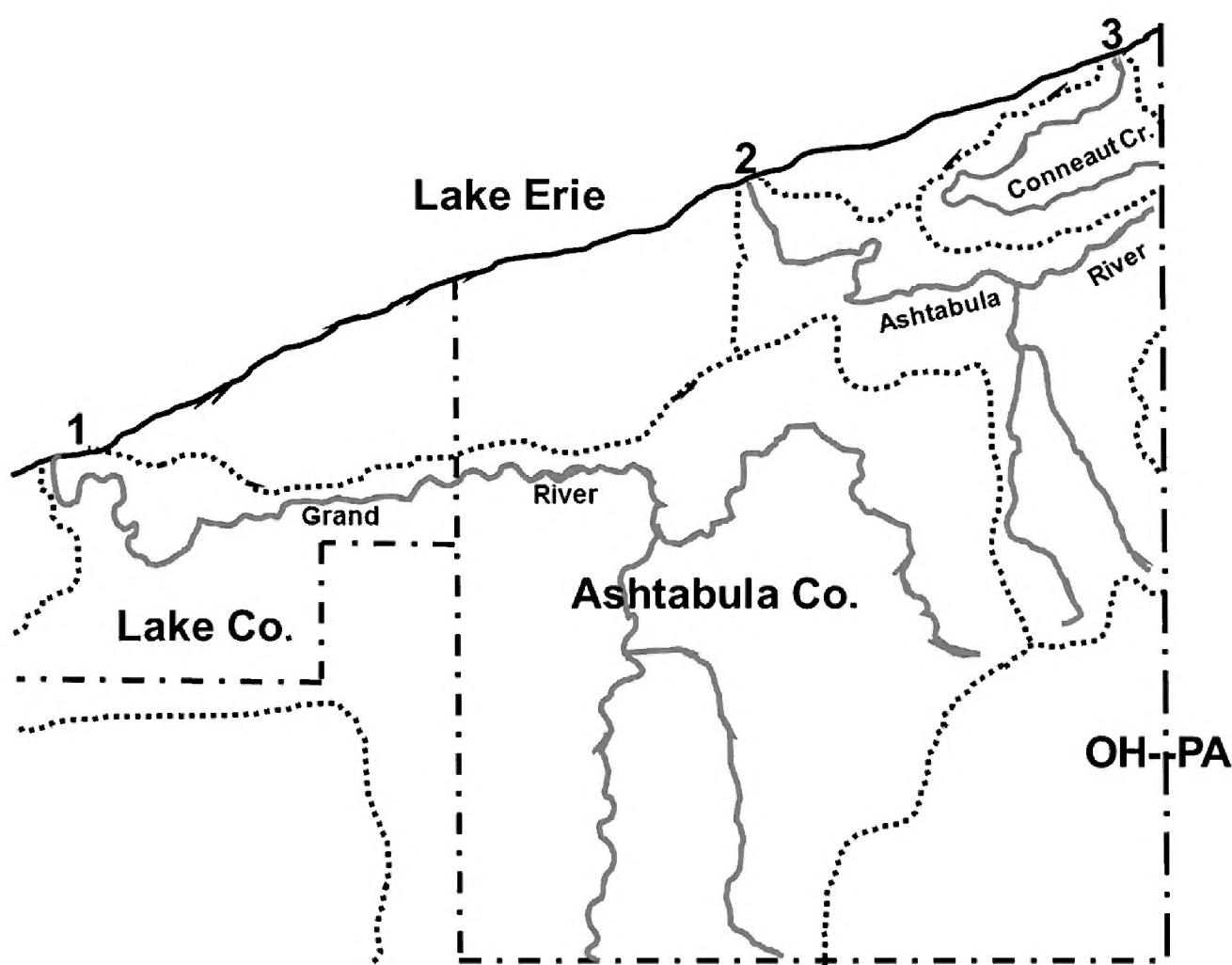


Figure 2. Map of Ashtabula County and eastern Lake County, showing the three drainage systems studied and their relative position in northeast Ohio: Grand River (1), Ashtabula River (2), Conneaut Creek (3). Blue lines represent the streams, dotted lines show the drainage demarcations, and alternating dot-dash lines represent county and state lines.

Results

First- or second-year tadpoles of *L. catesbeianus* were not present or were present in low numbers in the reaches of streams during some years when stream studies were conducted. At sites where only first-year tadpoles were captured, no tadpoles were found infected with adult female *L. cyprinacea*. However, it is probable that some first-year tadpoles were infected with earlier, microscopic stages of the parasite's life cycle on the gills or in the oral chamber, which would not be observable in the field. All mature *L. cyprinacea* were found on second- or, in a few cases (one of three), third-year tadpoles. The intensity of the infections and the prevalence of parasites are presented for sites within the reaches of the streams studied in Table 1. Most parasitized tadpoles were infected with one copepod; however, tadpoles with 2–7 copepods in various stages of development were not uncommon. Female *L. cyprinacea* were embedded posterior to the papillae of the mouth, in or near the groove of the body–tail juncture, or near the vent or the origin of the hind legs (Fig. 3). They also were attached near the spiracle, on the tail near its base, on the thigh, and on the distal end of the shank. Infected tadpoles frequently showed edema of the thigh and subdermal hemorrhaging around the site of anchor penetration; connective (scar) tissue encircling the external base of the mature female was present (Fig. 3). Tadpoles and metamorphosing individuals with parasites embedded near or at the tail base

or along its length often developed various degrees of tail curvature. Eight metamorphosing tadpoles exhibiting moderate to severe tail curvature were maintained in captivity in the laboratory until the tail had been completely resorbed; metamorphosis resulted in juvenile frogs that appeared normal and moved without impediment. Only one out of 1489 tadpoles was observed in the field to have a developmental abnormality (a J-shaped tail); that tadpole was apparently not parasitized by *L. cyprinacea*.

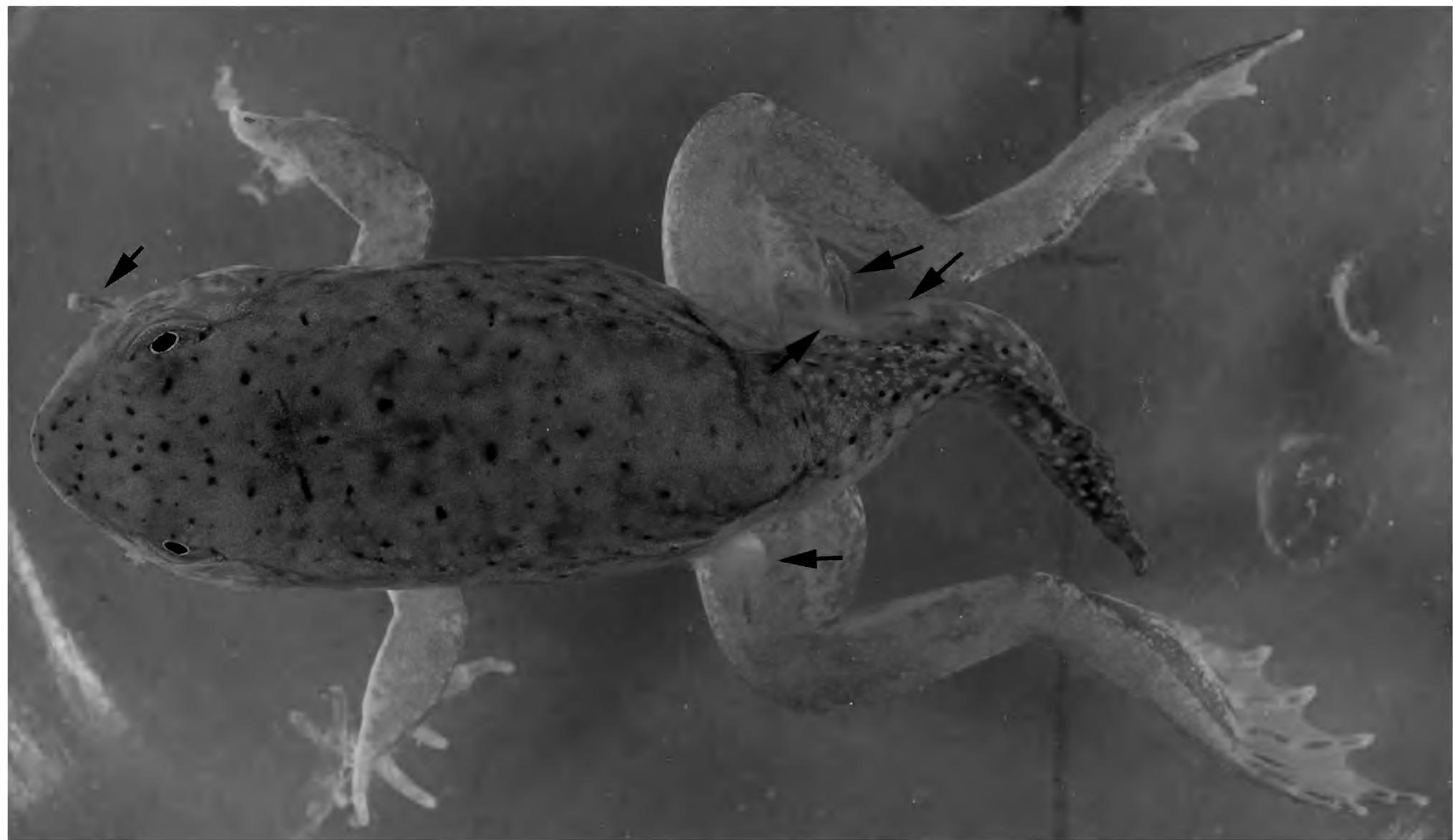


Figure 3. Metamorphosing *Lithobates catesbeianus* with five embedded Anchor Worms, showing observed tail curvature.

Discussion

These results represent the first report of *L. cyprinacea* infecting *L. catesbeianus* in northeastern Ohio streams. *Lernaea cyprinacea* is a thermophilic species, and its rate of development varies greatly with water temperature (Grabda 1963, Kupferberg et al. 2009, Shields 1978.). Although water temperatures at stream sites we sampled were not routinely recorded, variation in the intensity of the infection and prevalence of *L. cyprinacea* may be attributed to yearly differences in water temperature caused by modifications of stream discharge, precipitation patterns, or alterations of riparian zone vegetation that modify stream shading. The number of generations per year is dependent upon environmental temperature and changes with latitude and climate (Grabda 1963). Geographic expansion is likely in areas experiencing variable weather patterns with elevated seasonal temperatures from year to year (Plaul et al. 2010) or with global warming (Kupferberg et al. 2009). Therefore, prevalence of amphibian infections with *L. cyprinacea* could predictably increase.

Lernaea cyprinacea has been found to cause mortality in fishes (Enders and Rifenburgh 1928, Goodwin 1999, Shariff and Sommerville 1986). In amphibians, *L. cyprinacea* caused limb abnormalities in field-caught populations of *Hylarana chalconota* (Schlegel) in Indonesia (67.3% prevalence; Ming 2001) and in *Rana boylii* Baird of Northern California (9.2–10.5% prevalence; Kupferberg et al. 2009); it was associated with high mortality (32%) in farm-raised *A. mexicanum* in Brazil (Carnevia and Speranza 2003) and in *L. catesbeianus* in Brazil (Martins and Souza 1995). Developmental abnormalities of limbs or digits and mortality from *L. cyprinacea* infection were not noted in field populations of *Hyla pulchella cordobae* Barrio in Argentina (Alcalde and Batistoni 2005), *L. catesbeianus* in Argentina (Salinas et al. 2016), or *L. catesbeianus* in central Ohio (Tidd 1962). However, swelling of the thigh was noted in this study when parasites were imbedded either in the thigh or near its base. Curvature of the tails of tadpoles with embedded *Lernaea* has previously not been described. It was beyond the scope of this study to examine tadpoles for other species of parasites, but tail curvature could result from parasitism by other species and requires additional study. If severe, tail curvature may affect swimming performance and impede predator avoidance.

Table 1. Parasitic female *Lernaea cyprinacea* found attached to second-year tadpoles of *Lithobates catesbeianus* in three streams of northeastern Ohio. N¹ = number of first-year tadpoles; N² = number of second-year tadpoles. When N² = 0, no second-year tadpoles were found that year. When N¹ is blank, the number of first-year tadpoles was either 0 or not recorded.

Locality	River mile	Year	N ¹	N ²	Intensity of parasitism (no. of attached parasites)								Prevalence
					0	1	2	3	4	5	6	7	
Grand River													
Site 1	21.5	1993		106	87	19							17.9
		1997		106	103	3							2.8
		1999		3	2	1							33.3
		2002		1		1							100
		2008		0									0
		2009		0									0
		2010		114	7	16	35	33	17	5	1		93.9
		2011–2017		0									0
Ashtabula River													
Site 1	6.5	2005		119	44	38	29	4	1	2	1		63
		2014–2016		0									0
		2017		75	75								0
Site 2	21.1	2016	89	173	0								0
		2017		4	3	1							25
Conneaut Creek													
Site 1	22.3	2002		88	87	1							1.1
Site 2	17.7	1999		32	7	9	8	5	2	1			78.1
		2000		0									0
		2002		21	20	1							4.8
		2017		0									0
Site 3	5.4	2003		10	8	2							20
Site 4	14.3	1999		3	2	1							33.3
		2012		42	27	7	4	2	1	1			35.7
Site 5	22.8	2002		32 ^a	4	8	5	3	10	2			87.5
		2003	69	0	69								0
		2005		42	5	3	15	6	9	3	0	1	88.1
		2006		175	151	21	3						13.7
		2007		197 ^b	145	42	10						26.4
		2008		18	17	1							5.6
		2012		42 ^c	10	10	10	2	4	5	0	1	76.2
		2016–2017		0									0
Site 6	14.8	2002		2	1			1					100
		2005		11	1	5	2	3					90.9

^a16 additional first-year tadpoles of *L. clamitans* were examined, all without *L. cyprinacea*.

^bFour additional tadpoles of *L. clamitans* were examined, one with one *L. cyprinacea*.

^c10 additional first-year tadpoles of *L. clamitans* were examined, all without *L. cyprinacea*.

Tadpoles of *L. catesbeianus* in this study often showed subdermal hemorrhaging around the connective tissue surrounding the base of the attached female copepod. Development of the anchor associated with embedding and elongation of female *L. cyprinacea* was found to include not only the integument, but always affected some internal organ of *Lithobates pipiens* (Schreber) tadpoles (Tidd and Shields 1963). Tidd and Shields (1963) considered subdermal hemorrhaging and blood loss the probable causes of mortality of most tadpoles studied in the laboratory. Few to no *L. catesbeianus* tadpoles were captured at sampling sites in some years, and the population status of this species has not been monitored in these streams (Table 1). It is unclear if the infection of *L. catesbeianus* tadpoles by *L. cyprinacea* has an impact upon its status in these streams.

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Seasonal Diet Shifts in the Rusty Crayfish, *Faxonius rusticus* (Girard)

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Abstract: The rusty crayfish, *Faxonius rusticus* (Girard), is a species of great management concern due to its rapid range expansion across the northeastern United States and southern Canada. The rusty crayfish is an opportunistic omnivore that has been shown to disrupt invaded ecosystems by outcompeting native crayfish species for food and shelter. While numerous studies have demonstrated the community and ecosystem effects of rusty crayfish invasions, relatively little attention has been given to understanding the species' ecological needs, especially in their native range. In particular, relatively little is known about their foraging habits within their native range. This study aims to elucidate the feeding habits of rusty crayfish within their native range of the Ohio River Valley to determine (1) the most commonly consumed food items, and (2) how the diet of the species changes across seasons. Results show seasonal shifts in the diets of the rusty crayfish that likely coincide with seasonal abundance of food resources. Detritus was the dominant food item across months of study. The percent contribution of animal tissue and plants to the diets varied seasonally. The results elucidate the plasticity of rusty crayfish diets, which may be an important contributor to their success as an invasive species.

Keywords: diet, foraging, invasive species, crayfish

Introduction

The rusty crayfish (*Faxonius rusticus*) has undergone rapid range expansion in recent decades (Olden et al. 2006). While the species is native to the Ohio River Valley, its range has expanded to include much of the northeastern United States and Canada, including the Great Lakes region (Olden et al. 2006). The surprising rate of the species' range expansion, paired with the major ecological effects of its introduction (Gherardi 2007; Lodge et al. 1987, 1994; Olden et al. 2006; Morse et al. 2013) has made rusty crayfish a species of great management concern. Rusty crayfish are commonly used by anglers as live bait, and their range expansion is linked to the release of unused baits in waterbodies outside of the natural range of the species (Lodge et al. 2000, Ludwig and Leitch 1996). Therefore, management efforts to date have focused on lowering the rate of introductions and using intensive trapping mechanisms to lower population sizes in invaded areas. These efforts have remained largely ineffective at lowering non-native rusty crayfish populations (Lodge et al. 2000).

The ecological effects of rusty crayfish on invaded habitats have been well documented. Rusty crayfish invasions decrease population sizes of native crayfish species (Lodge et al. 2000), disrupt food webs (Lodge et al. 1994), and lead to a general loss of biodiversity (Lodge and Lorman 1987). While numerous studies elucidate the ecological effects of this species, less attention has been given to understanding the characteristics that make this species such a successful invader (Gherardi 2007). Behavioral studies have focused on understanding the competitive dynamics between rusty crayfish and native crayfish species (e.g., Pintor et al. 2008), and genetic studies have focused on understanding hybridization dynamics that may accelerate the decline of native crayfish populations (e.g., Perry et al. 2001). However, the plasticity of their diet has received comparatively little attention as a plausible mechanism permitting rusty crayfish range expansion.

The northern range expansion of rusty crayfish has introduced the species to more extreme seasonal weather patterns than it experiences in its native range (e.g., northern Wisconsin versus southern Ohio). Consequently, this exposes rusty crayfish to new metabolic and foraging challenges that must be overcome to survive and reproduce. In order to survive in areas with seasonal shifts in temperature and resource abundance, organisms must exhibit considerable plasticity in their diets and be able to utilize a variety of nutritional resources to meet their physiological demands (Ebling and Barrett 2008, Heng et al. 2018). In particular, organisms in these seasonally variable environments must shift their diet to match the availability of seasonally abundant food resources (Correia 2002). Rusty crayfish are opportunistic omnivores that can feed on a variety of food items across trophic levels (Roth et al. 2006). Thus, it is reasonable to predict that rusty crayfish can undergo seasonal diet shifts to match their resource use to the available resources in their environment. This study aims to elucidate the use of food resources

by rusty crayfish across seasons. We tested the hypothesis that rusty crayfish exhibit seasonal variation in their diet by analyzing the gut contents of specimens collected monthly. The study was conducted in the native range of the species in the Ohio River Valley to better understand their natural foraging habits as they relate to the invasion success of the species and their overall ecological impacts on invaded regions (Gherardi 2007). Additionally, understanding the feeding habits of rusty crayfish in their native range will allow managers to better identify habitats susceptible to invasions and develop more effective trapping strategies (Olden et al. 2006).

Methods

Specimen Collection. Male and female rusty crayfish were collected by hand netting from Sharon Creek (Sharonville, Ohio, USA) monthly from April–November 2016. Due to the inactivity of the animals at low environmental temperatures and frozen water surfaces, collections were unsuccessful at capturing a sufficient number of samples in colder winter months (December–March).

Upon collection, specimens were placed in an ice bath to lower their metabolic rates and slow the progress of food through the alimentary canal. After collection, specimens were immediately transported to the lab and euthanized in a laboratory freezer (approximately -20°C), where they remained frozen until analysis. Only non-molting individuals were analyzed for stomach contents. Sample sizes for analyses were N = 16 (April), N = 15 (May, June, and July), N = 14 (August), N = 12 (September), N = 11 (October), and N = 8 (November).

Gut Content Analysis. Immediately prior to dissection, specimens were removed from the freezer and thawed to room temperature. Morphometric measurements including wet mass, carapace length, and cephalothorax length were measured for each animal. Animals were sexed by visual examination of the reproductive organs. The stomachs were removed by dissection and placed in a 2 mL snap-cap tube containing 0.5 mL of distilled water. The stomachs were ruptured manually using a dissection probe so that the contents could be removed and homogenized by mixing by hand with the dissection probe; 0.25 mL of homogenized stomach contents was placed on a microscope slide and viewed at 100X total magnification. All identifiable material in the stomach contents was classified into one of the following categories: detritus, animal tissue, plant tissue, algae, diatoms, cyanobacteria, and protozoa. Once all identifiable material within the visual field was categorized, the stage of the microscope was moved to create a new visual field, and the material within that field was identified. This procedure was repeated until ten total visual fields were inspected for each specimen. The frequency of each food item was tallied across the ten visual fields. 400X total magnification was used to help identify contents when needed. To determine the percent contribution of each food item to the diet of the crayfish each month, the total tally of each food category was divided by the total number of identifiable food items found in that month and multiplied by 100.

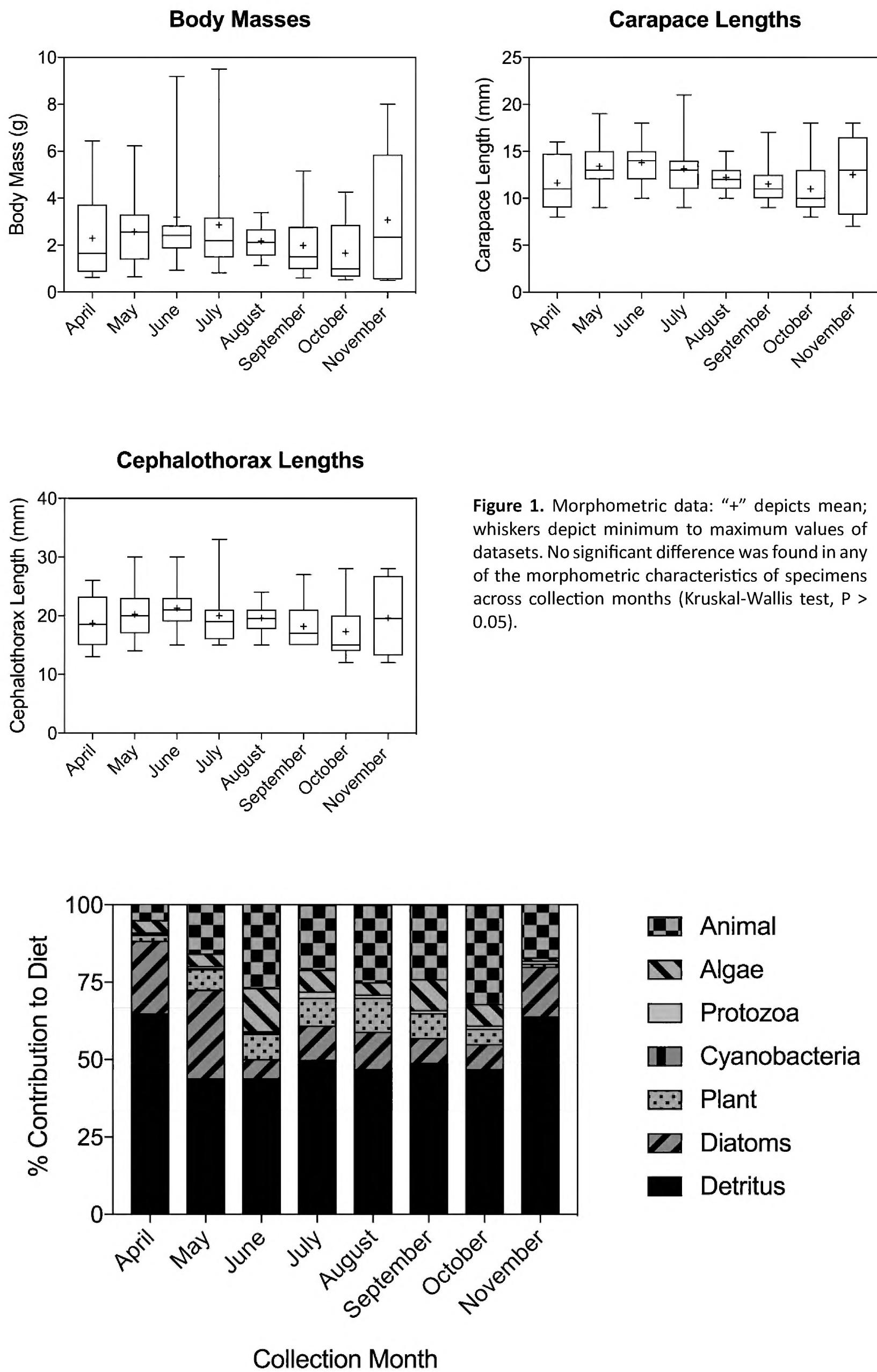
Statistical Analysis. Kruskal-Wallis tests were used to test for differences in morphometric characteristics of the specimens across months. Chi-squared analysis was used to determine whether the proportions of food materials in the stomachs varied across months. A Pearson's correlation was used to test the *a posteriori* hypothesis of correlation between the contributions of animal tissue and diatoms across months.

Results

No significant differences were found among monthly collections for body mass ($H = 7.691$, $df = 7$, $P > 0.05$), carapace length ($H = 13.65$, $df = 7$, $P > 0.05$), or cephalothorax length ($H = 9.768$, $df = 7$, $P > 0.05$; Figure 1). The percent contributions of diet items in the guts of rusty crayfish varied significantly by collection month ($X^2 (35) = 97.05$, $P < 0.0001$; Figure 2). Detritus was the most abundant food item in the guts across months, ranging from 44–65% of the overall diet. Cyanobacteria and protozoa were not commonly found in the gut contents. Plant material and algae were found in all collection months, but contributed a relatively small proportion to the overall diet of the crayfish. The abundance of both plant material and algae in the guts increased during summer months and decreased during fall and early spring months. Interestingly, the abundance of diatoms and animal tissue were negatively correlated across months ($R^2 = 0.8722$, $P < 0.001$; Figure 3). When diatom contributions to the diet were highest in spring months, animal contributions to the diets were their lowest. When the frequency of animal content peaked during summer and early fall months, diatom contributions to the diets decreased.

Discussion

Because of the opportunistic, omnivorous nature of their feeding, we expected rusty crayfish to exhibit varied diets that



incorporate a number of food resources across trophic levels. The ability to utilize a variety of food items has been suggested as an important determinant of range expansion and invasion success (Vázquez 2006). Species that are able to consume a variety of food resources are more likely than specialists to encounter suitable food resources in non-native habitats (Vázquez 2006). Expansion into non-native habitats increases the likelihood of an organism encountering novel food resources, and omnivorous crustaceans have been shown to readily track and consume novel foods (Tran 2015). The ability to shift diets seasonally within their natural range of the Ohio River Valley suggests a high level of plasticity in the diet that likely benefits rusty crayfish during their invasion of new habitats.

The food resources consumed by rusty crayfish in our study were consistent with those consumed by other crayfish species (Parkyn et al. 2001, Whiteledge and Rabeni 1997).

Our data suggest that rusty crayfish vary their diets to match seasonally abundant food resources. For example, aquatic insect larvae emerge during late spring and early summer in the Ohio River Valley (DeWalt et al. 2016), and this corresponds with the increase in animal tissue abundance in the stomachs of rusty crayfish during summer. In our study, April had the lowest percent contribution of animal tissue to the diets of rusty crayfish. It is possible that this was caused by the lack of animal tissue resources for consumption prior to events such as the emergence of aquatic insect larvae.

The negative correlation between the percent contributions of diatoms and animal tissue to the diets of rusty crayfish was an unexpected result, but shows the ability of rusty crayfish to feed at different trophic levels. Our data suggests that rusty crayfish may increase their diatom consumption in response to low environmental abundance of animal tissue for consumption. Morphological studies have confirmed that crayfish have the oral appendage structure necessary for filter-feeding (Budd et al. 1977). It is unknown whether rusty crayfish are ingesting these diatoms by filter-feeding or through the consumption of other food items on which the diatoms are found. However, evidence suggests that other crustaceans (e.g., copepods) show strong growth efficiency when fed a diet including diatoms (Jones and Flynn 2005). Thus, it is plausible that rusty crayfish are supplementing their diets with diatoms ingested through filter-feeding during certain times of the year. This ability to supplement their diet with less nutritionally desirable foods may allow for better invasion potential in rusty crayfish as they encounter novel food resources in spatially and temporally variable habitats.

Previous research on rusty crayfish has focused extensively on the ecological impacts of their invasion. However, more attention should be given to studying this species in their native range to better understand the evolutionary and ecological pressures that have shaped their resource needs and behaviors. In particular, more studies comparing the behaviors and ecological roles of rusty crayfish in native versus invaded ranges are needed (Gherardi 2007). By doing so, researchers will better understand how the plasticity of traits influences the invasion potential of species and how successful invaders cope with the ecological pressures of newly invaded habitats.

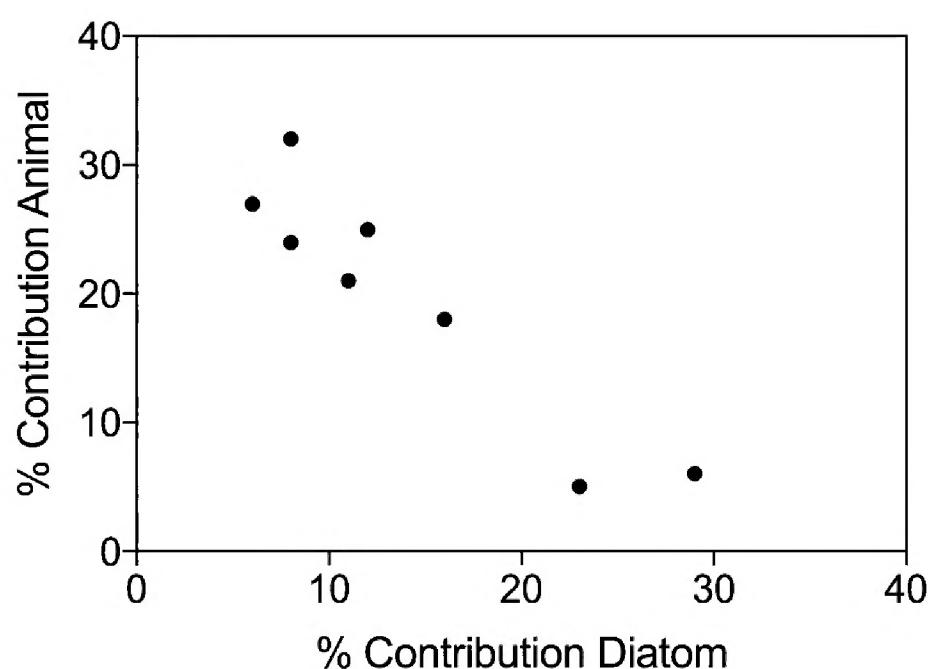


Figure 3. Correlation between diatom and animal tissue abundances. Pearson $R^2 = 0.08722$, $P < 0.001$.

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